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COMPARATIVE STUDIES ON THE OPTIMIZATION OF STAPHYLOKINASE AND NATTOKINASE PRODUCED FROM STAPHYLOCOCCUS AUREUS AND BACILLUS INAQUSORUM

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ABSTRACT

Fibrinolytic enzymes are widely used as thrombolytic agents. The present study is based on comparative studies of the fibrinolytic enzymes produced by *Staphylococcus aureus* and *Bacillus inaqusorum*. The result of β-hemolytic activity, proteolytic activity, invitro clot lysis, anti-thrmobotic activity and the study of different production parameter showed that the Staphylokinase enzymes is more efficient and effective as compared to Nattokinase. The statistical analysis was carried out using one factor ANOVA test to know the significant effect of the studied parameter on the enzyme production and it showed that the entire factor showed significant difference as p value was ≤ 0.05 except for the effect of inhibitor used in the study was not much significant. The reasonal difference among the three population means was further confirmed by tukey method.

KEYWORDS: Cardiovascular Disease, Fibrinolytic, Staphylokinase (SAK), Thrombolytic Assay and Fibrin

1. INTRODUCTION

One of the reasons for cardiovascular disease is thrombosis. Thrombosis occurs due to formation of clots in the arterial system. Fibrionolytic enzymes are used to treat thrombosis. Fibrinolytic enzymes convert plasminogen to plasmin and lyse clots by breaking down the fibrin contained in the clot. Several fibrinolytic enzymes such as Staphylokinase, Nattokinase, and Streptokinase obtained from different organisms such as *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp. etc. respectively (Kim et al., 2000). Fibrinolytic protease is well-known as a sub class of protease, which has an ability to degrade fibrin (Fujita, M. et al., 1993). Staphylokinase is an ideal fibrin specific plasminogen activator that converts plasminogen to plasmin which in turn attacks on the fibrin clots. SAK possess better fibrin specificity than t-PA and are capable of dissolving platelets-rich clots (Collen, D. et al., 1993, Collen, D. et al., 1992). Staphylokinase is an extracellular protease of 136 aminoacid and is a 15kDa protein, produced during the late exponential phase by lysogenic strain of *Staphylococcus aureus* (Jasim, H. et al., 2015).

SAK has good fibrinolytic activity, dissolve plasminogen into plasmin thus acting as efficient clot buster, but it is quite expensive because some strains of *Staphylococcal* spp. are pathogenic and hence the enzyme has to be expressed in other host system (Lack, C. 1987). Whereas Nattokinase is an enzyme considered to be a promising remedy for thrombosis due to its presence in food and robust fibrinolytic activity. Nattokinase is an enzyme extracted and purified from a Japanese food called natto. Natto is made from fermented soyabeans. It is produced by fermentation by adding the bacterium *Bacillus natto* which is the preeminent nattokinase producer (Haritha, M. et al., 2011). It is a serine protease of the subtilisin family with 275 amino acid residues and molecular weight of 27,728 Dalton having potent fibrinolytic activity (Mohansrinivasan, V. et al., 2014). Nattokinase is in expensive and promote normal blood pressure, reduce whole blood viscosity and increase circulation being an effective supplement to support cardiovascular disease (Kim, W. et al., 1996).

2. MATERIALS AND METHODS

A. Sample Collection and Revival of culture: Isolates were obtained from the previous studies done which were already sequenced. The isolates were revived on nutrient agar plates. The colony, morphological characteristics were noted down and gram staining was performed. All the further experiment was done in triplicates.

B. β-Haemolytic Activity: *Staphylococcus aureus* and *Bacillus inaqusorum* were screened on blood agar plate for studying β-haemolytic activity by spot inoclutaion. The plates were then incubated at 37°C for 24 hours and next day zone of blood lysis was observed and measured by zone reader.

C. Proteolytic Activity: Proteolytic activity was observed by doing spot inoculation of *Staphylococcus aures* and *Bacillus inaqusorum* on gelatin agar plates. The plates were then incubated at 37°C for 24 hours and next day zone of gelatin hydrolysis was observed and measured by zone reader.

D. Production Medium: The *Staphylococcus aureus* was grown in production medium containing(gm/L) nutrient broth(10), yeast extract(3), NaCl(5), glycerol(10) pH 7.2 at 37°C for 24 hours and *Bacillus inaqusorum* was grown in production medium containing(gm/L) soyapeptone(10), K_2HPO_4(2), $MgSO_4$(1), maltose(20), yeast extract(10), glucose(29), pH 7.2 at 37°C for 24 hours.

E. Crude Enzyme Preparation: After 24hours, 10ml of sample from the production medium was withdrawn aseptically in sterile centrifuge tube and was centrifuged at 2000 rpm for 20min at 4°C. The supernatant was

considered as crude enzyme and was further used to study invitro clot lysis and anti-thrombolytic assay.

F. Invitro Clot Lysis: The weight of empty appendrof tubes was taken (W_1). 1ml of blood withdrawn from healthy volunteer was transferred on to an appendrof tubes and the blood was allowed to clot at 37°C for 1 hour. After 1 hour, serum was removed and again the weight of appendrof tubes with blood clot was measured (W_2). Then 1ml of crude enzyme was added to the appendrof tubes and incubated at 37°C for 90 min. After incubation the lysed part was discarded and the weight of blood clot measured (W_3). For control, 1ml of blood was taken into an appendrof, allowed to clot at 37°C for 1 hour, serum was removed and 1ml of sterile production broth was added and incubated at 37°C for 90 min. The percentage of clot lysis was calculated for control tubes as well as tubes with crude enzyme by using the formula:

% of Clot Lysis: 100-{(W3-W1) / W2-W1)} X 100

G. Anti-thrombotic Assay: Whole blood was collected from healthy volunteer. 100µl of whole blood was taken in an appendrof tubes and 100 µl crude enzyme was added. The tubes were incubated at 37°C for 90min. After incubation the time required for the blood to clot was noted and compared with control which was containing only 100µl of whole blood.

H. Optimization study of production parameter on Staphylokinase and Nattokinase production: Different parameter like Incubation time, Temperature, pH, Carbon source and Nitrogen sources were studied to observe the effect on Staphylokinase and Nattokinase production. The parameters were optimized by varying one factor, while keeping the other parameters constant. The entire optimization procedure was carried out in triplicates and the result was recorded. After incubation, the medium was centrifuged at 7000 r.p.m for 20 minutes at 4°C. The supernatant obtained was proceeded for invitro clot lysis and anti-thrombotic assay (Mukesh, D. et al., 2013).

1. Effect of Incubation time: To study the effect of incubation time on Staphylokinase and Nattokinase production, Individual production medium was inoculated with selected isolates and the flasks were then incubated for 4 days and invitro clot lysis and anti-thrombtic activity was studied after 24, 48, 72 and 96 hours of incubation.
2. Effect of Temperature: To study the effect of temperature on Staphylokinase and Nattokinase production, Individual production medium was inoculated with selected isolates and incubated at previously optimized time at 8°C, Room temperature, and 37°C.
3. Effect of pH: To study the effect of pH on Staphylokinase and Nattokinase production, medium with different pH (5, 7 and 9) was inoculated with respective isolates and incubated at previously optimized time and temperature
4. Effect of Carbon Sources: To study the effect of carbon source on Staphylokinase and Nattokinase production, medium with above optimized parameters containing different carbon sources (Glucose, Lactose and Sucrose) were inoculated with respective isolates and incubated at previously optimized time and temperature.
5. Effect of Nitrogen Sources: To study the effect of nitrogen source on Staphylokinase and Nattokinase production, medium with above optimized parameters containing different nitrogen sources (Beef Extract, Casein and peptone/soypeptone) were inoculated with respective isolates and incubated at previously optimized time and temperature.
6. Effect of Activators: To study the effect of activator on Staphylokinase and Nattokinase production, medium with above optimized parameters containing different activators (MgSO4, MnSO4 and FeSO4) were inoculated with respective isolates and incubated at previously optimized time and temperature.
7. Effect of Inhibitors: To study the effect of Inhibitors on Staphylokinase and Nattokinase production, medium with above optimized parameters containing different Inhibitors (SDS, EDTA and AgNO3) were inoculated with respective isolates and incubated at previously optimized time and temperature.
8. Comparision between Staphylokinase and Nattokinase Production Before and After Optimization: The medium was prepared according to the optimized result for both the enzyme and was incubated at optimum conditions. After incubation, incitro clot lysis and anti-thrombotic activity was performed and the results were compared with unoptimized media results.

I. Statistical Analysis: The results obtained for the optimization of Staphylokinase and Nattokinase production was statistically analysed using standard deviation and one factor-ANOVA in Microsoft excel. The significance was further tested by Tukey methods using studentized range distribution table.

3. RESULTS AND DISCUSSION

A. Sample Collection: Both the isolates were grown on nutrient agar, Staphylokinase appeared Gram positive cocci occurring singly or in cluster and Nattokinase appeared Gram positive long rods occurring singly or in chain.

B. β-Haemolytic assay: S-90 and N-15 showed β-haemolysis on blood agar and the zone size measures was 24mm and 18mm respectively as shown in fig 1.

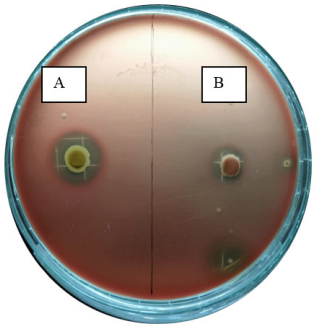


Fig 1: β-Haemolytic Activity of A) Staphylokinase and B) Nattokinase on Blood Agar Plates

C. Proteolytic Assay: Both the isolates obtained showed proteolytic activity on gelatin agar plate and the zone size measured was 18mm for Staphylokinase and 16mm for Nattokinase as shown in fig 2.

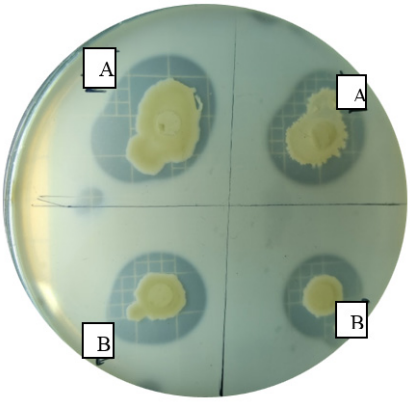


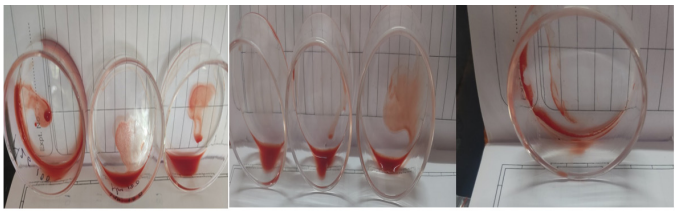
Fig 2: Proteolytic Activity of A) Staphylokinase and B) Nattokinase on Gelatin Agar Plates

D. In Vitro Clot Lysis: The result of in vitro clot lysis revealed that Staphlyokinase showed 32% and Nattoinase showed 23% of clot lysis respectively as compared to control which did not show any lysis, as shown in fig 3.

(A) (B)

Fig 3: In Vitro Clot Lysis of A) Staphylokinase and B) Nattokinase

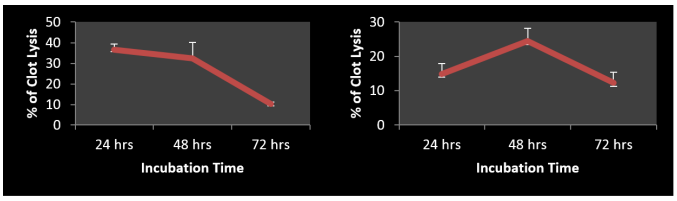
E. Anti-thrombotic assay: The result of anti-thromobotic assay showed that Staphylokinase and Nattokinase showed strong anti-thrombolytic affect with 100µl of crude enzyme as compared to control as shown in fig 4.The consistency of blood showed that the addition of enzymes did not allow the blood to clot reflecting its anti-thrombotic property



(A) (B) (C)

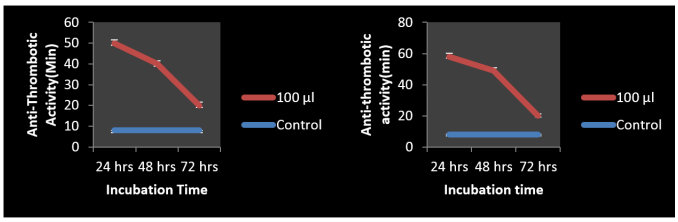
Fig 4: Anti-Thrombotic Property of A) Staphylokinase B) Nattokinase C) Control

F. Optimization of Production Parameters: Effect of Incubation time: The optimum incubation time for invitro clot lysis and anti-thrombotic activity increased for Staphylokinase at 24hrs, while that of Nattokinase at 48hrs and then the enzyme activity decreased rapidly as shown in fig 5. The Statiscal analysis by ANOVA showed that there is significant difference incubaton time on enzyme production for both the eznyme as p value is less than 0.05. Further analysis by Tukey method revealed that all the three incubation time are significantly different at α=0.05 as shown in table 1 and table 2.
*For Tukey method **T=1.9368**.



(A) (B)

Fig 5: Result of Effect of Incubation on Invitro Clot lysis of (A) Staphylokinase and (B) Nattokinase



(A) (B)

Fig 6: Result of Effect of Incubation Time on Anti-thrombotic activity of (A) Staphylokinase and (B) Nattokinase

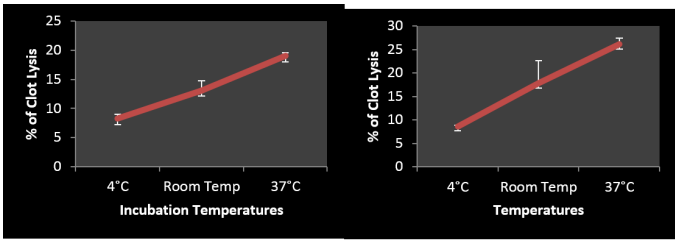
Table 1: ANOVA to Study Effect of Incubation Time on Enzyme Production

Staphylokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1192.142	2	596.0711	17.81503	0.002994	5.143253
Within Groups	200.7533	6	33.45889			
Total	1392.896	8				

Nattokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	248.7489	2	124.3744	7.634497	0.02245	5.143253
Within Groups	97.74667	6	16.29111			
Total	346.4956	8				

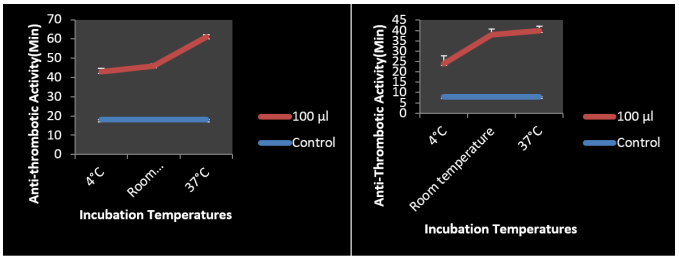
Table 2: Tukey Method for Enzyme Production			
Staphylokinase			
X1 -X2	9.6	9.6>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	2.633333	2.63>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	12.23333	12.23>1.9368	difference between 2 and 3 is sig at α=0.05
Nattokinase			
X1 -X2	4.266667	4.26>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	26.26667	26.26>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	22	22>1.9368	difference between 2 and 3 is sig at α=0.05

2. Effect of Temperature: The maximum clot lysis and anti-thrombotic activity was observed at 37°C temperature for Staphylokinase and Nattokinase as shown in fig 7 and fig 8. The Statiscal analysis by ANOVA showed that there is significant difference incubaton temperature on enzyme production for both the enzyme as p value is less than 0.05. Further analysis by Tukey method revealed that all the three incubation temperature are significantly different at α=0.05 as shown in table 3 and table 4.



(A) (B)

Fig 7: Result of Effect of Temperature on Invitro clot lysis of (A) Staphylokinase and (B) Nattokinase



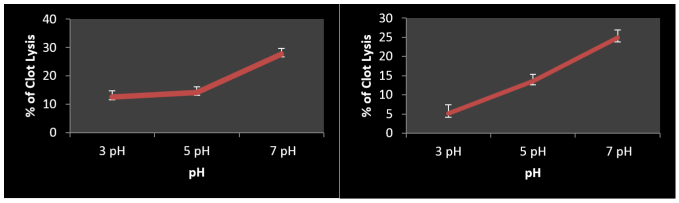
(A) (B)

Fig 8: Result of Effect of Temperatures on Anti-thrombotic activity of (A) Staphylokinase and (B) Nattokinase

Table 3: ANOVA to Study the Effect of Incubation temperature on Enzyme Production						
Staphylokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	175.46	2	87.73	48.92007	0.000193	5.143253
Within Groups	10.76	6	1.793333			
Total	186.22	8				
Nattokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	458.0022	2	229.0011	18.4728	0.002727	5.143253
Within Groups	74.38	6	12.39667			
Total	532.3822	8				

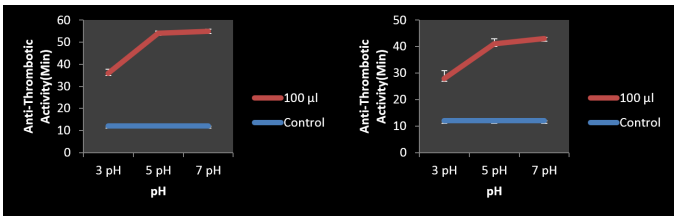
Table 4: Tukey Method for Enzyme Production			
Staphylokinase			
X1 -X2	4.9	4.9>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	10.8	10.8>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	5.9	5.9>1.9368	difference between 2 and 3 is sig at α=0.05
Nattokinase			
X1 -X2	9.166667	9.16>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	17.46667	17.4>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	8.3	8.3>1.9368	difference between 2 and 3 is sig at α=0.05

3. Effect of pH: The maximum clot lysis and anti-thrmobotic activity was seen at pH 7 for Staphylokinase and Nattokinase as shown in fig 8 and fig 9. The Statiscal analysis by ANOVA showed that there is significant effect of pH on enzyme production for both the enzyme as p value is less than 0.05. Further analysis by Tukey method revealed that for Staphylokinase there was no significant difference between pH 3 and pH 5 , wherease there was significant difference between pH 5 and pH 7 and pH 7 and 3. But for Nattokinase analyisis by Tukey method revelaed the all the 3pH used in the experiment are significantly different at α=0.05 as shown in table 5 and table 6.



(A) (B)

Fig 8: Result of Effect of pH on Invitro clot lysis of (A) Staphylokinase and (B) Nattokinase



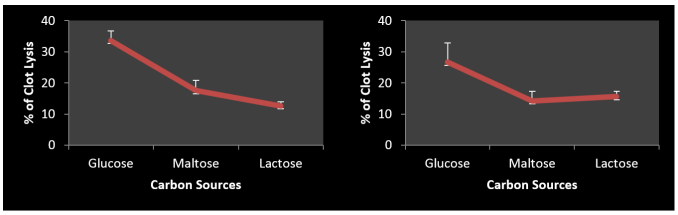
(A) (B)

Fig 9: Result of Effect of pH on Anti-Thrombotic Activity(Min) of (A) Staphylokinase and (B) Nattokinase

Table 5: ANOVA to Study the Effect of pH on Staphylokinase Production						
Staphylokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	419.3622	2	209.6811	32.86538	0.000585	5.143253
Within Groups	38.28	6	6.38			
Total	457.6422	8				
Nattokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	588.0267	2	294.0133	45.87587	0.000231	5.143253
Within Groups	38.45333	6	6.408889			
Total	626.48	8				

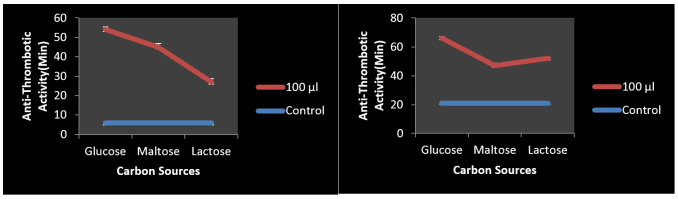
Table 6:Tukey Method for Enzyme Production			
Staphylokinase			
X1 -X2	1.566667	1.566<1.9368	difference between 1 and 2 is not significant
X1-X3	15.2	15.2>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	13.63333	13.63>1.936	difference between 2 and 3 is sig at α=0.05
Nattokinase			
X1 -X2	8.466667	8.46>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	19.73333	19.73>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	11.26667	11.26>1.9368	difference between 2 and 3 is sig at α=0.05

4. Effect of Carbon Sources: The maximum Staphylokinase production and anti-thromobotic activity was obtained with medium containing carbon source as Glucose>Maltose>Lactose. Similarly maximum Nattokinase production and anti-thromobotic activity was obtained with medium containing carbon source as Glucose>Lactose>Maltose as shown in fig 10 and fig 11. The Statiscal analysis by ANOVA showed that there is significant difference of carbon sources on Staphylokinase and Nattokinase production as p value is less than 0.05. Further analysis by Tukey method revealed that all the three carbon sources are significantly different at α=0.05 for Staphylokinase, but for Nattokinase it was studies that there is no significant difference between maltose and lactose, while glucose-lactose and glucose-maltose showed significant difference at α=0.05 as shown in table 7 and table 8.



(A) (B)

Fig 10: Result of Effect of Carbon Sources on Invitro clot lysis of (A) Staphylokinase and (B) Nattokinase



(A) (B)

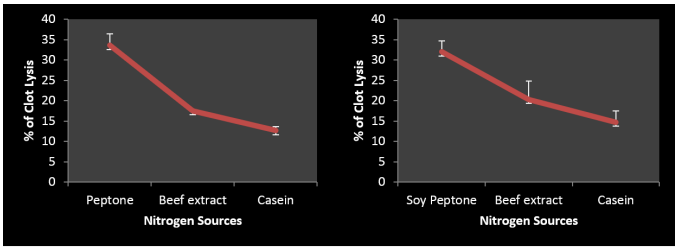
Fig 11: Result of Effect of Carbon Sources on Anti-Thrombotic Activity of (A) Staphylokinase and (B) Nattokinase

Table 7: ANOVA to Study the Effect of Carbon Sources on Enzyme Production						
Staphylokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	721.7489	2	360.8744	32.71754	0.000593	5.143253
Within Groups	66.18	6	11.03			
Total	787.9289	8				
Nattokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	276.72	2	138.36	5.381564	0.045855	5.143253
Within Groups	154.26	6	25.71			
Total	430.98	8				

Table 8: Tukey Method for EnzymeProduction			
Staphylokinase			
X1 -X2	1.566667	1.566<1.9368	difference between 1 and 2 is not significant
X1-X3	15.2	15.2>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	13.63333	13.63>1.936	difference between 2 and 3 is sig at α=0.05
Nattokinase			
X1 -X2	8.466667	8.46>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	19.73333	19.73>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	11.26667	11.26>1.9368	difference between 2 and 3 is sig at α=0.05

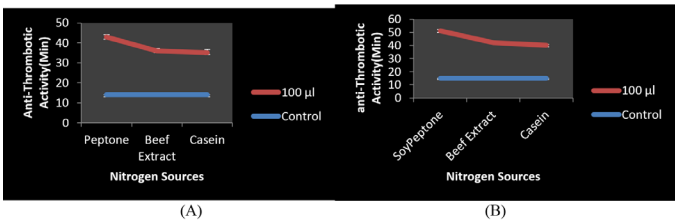
5. Effect of Nitrogen Sources: The maximum enzyme and anti-thrombotic activity for Staphylokinase and Nattokinase was obtained in the medium containing Peptone and Soypeptone as nitrogen source respectively as shown in fig 12 and fig 13. The Statiscal analysis by ANOVA showed that there is

significant difference of nitrogen sources on Staphylokinase and Nattokinase production as p value is less than 0.05. Further analysis by Tukey method revealed that all the three incubation temperature are significantly different at α=0.05 as shown in table 9 and table 10.



(A) (B)

Fig 12: Result of Effect of Nitrogen Sources on Invitro clot lysis of (A) Staphylokinase and (B) Nattokinase



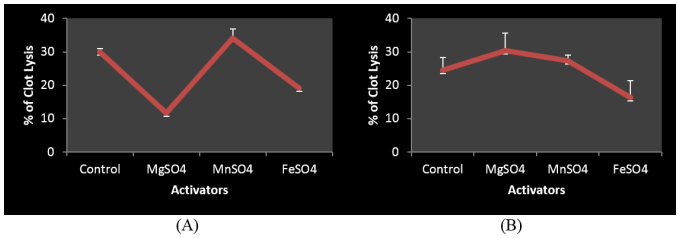
(A) (B)

Fig 13: Result of Effect of Nitrogen Sources on Invitro clot lysis of (A) Staphylokinase and (B) Nattokinase

Table 9: ANOVA to Study the Effect of Nitrogen Sources on Staphylokinase Production						
Staphylokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	379.5089	2	189.7544	41.32083	0.00031	5.143253
Within Groups	27.55333	6	4.592222			
Total	407.0622	8				
Nattokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	466.0156	2	233.0078	13.44793	0.006068	5.143253
Within Groups	103.96	6	17.32667			
Total	569.9756	8				

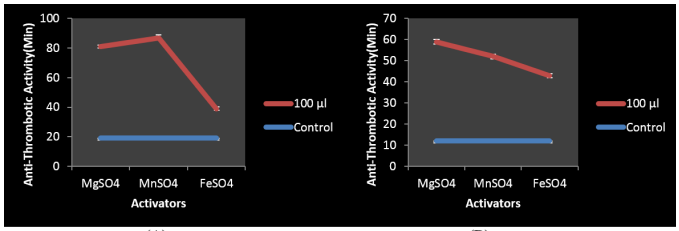
Table 10: Tukey Method for Enzyme Production			
Staphylokinase			
X1 -X2	8.333333	8.33>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	15.9	15.9>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	7.566667	7.56>1.9368	difference between 2 and 3 is sig at α=0.05
Nattokinase			
X1 -X2	11.7	11.7>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	17.26667	17.26>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	5.566667	5.56>1.9368	difference between 2 and 3 is sig at α=0.05

6. Effect of Activators: The maximum clot lysis and anti-thromobotic activity of Staphylokinase was observed when manganese sulphate was used as activator in the medium as compare to control which was not having activator. For Nattokinase maximum clot lysis and anti-thromobotic activity was observed when magnesium sulphate was used as activator in the medium as compare to control as shown in fig 14 and fig 15. Statistical analysis by ANOVA and Tukey method shpwed that there is significant difference between the activators on Staphylokinase and Nattokinase production at α=0.05 as shown table 11 and table 12.



(A) (B)

Fig 14: Result of Effect of Activators on Invitro clot lysis of (A) Staphylokinase and (B) Nattokinase



(A) (B)

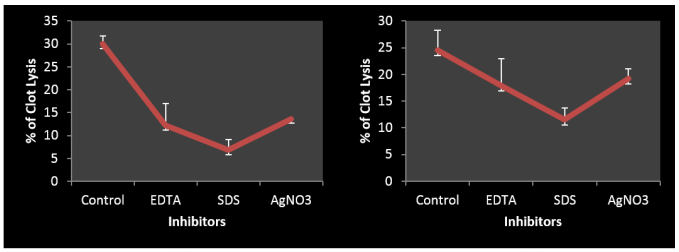
Fig 15: Result of Effect of Activators on Anti-Thrombotic Activity of (A) Staphylokinase and (B) Nattokinase

Table 11: ANOVA to Study the Effect of Activators on Enzyme Production						
Staphylokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	785.0022	2	392.5011	81.54455	0.000044	5.143253
Within Groups	28.88	6	4.813333			
Total	813.8822	8				
Nattokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	327.6689	2	163.8344	5.884153	0.038505	5.143253
Within Groups	167.06	6	27.84333			
Total	494.7289	8				

Table 12: Tukey Method for Enzyme Production			
Staphylokinase			
X1 -X2	22.46667	22.46>1.9368	difference between 1 and 2 is sig at α=0.05

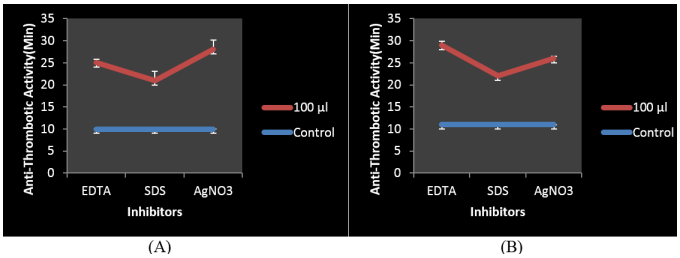
X1-X3	7.5	7.5>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	14.96667	14.96>1.9368	difference between 2 and 3 is sig at α=0.05
Nattokinase			
X1 -X2	3	3>1.9368	difference between 1 and 2 is sig at α=0.05
X1-X3	14.03333	14.03>1.9368	difference between 1 and 3 is sig at α=0.05
X2-X3	11.03333	11.03>1.9368	difference between 2 and 3 is sig at α=0.05

7. Effect of Inhibitors: For Staphylokinase the property of invitro clot lysis and anti-thrombotic activity decreased drastically when SDS was added into the production medium, even EDTA and silver nitrate showed decrease in enzyme activity as compared to control. Similarly for Nattokinase enzyme activity decreased in the presence of SDS as compared to control. EDTA and silver nitrate showed almost same amount of decrease in the enzyme activity as shown in fig 16 and fig 17. The statistical analysis done by ANOVA showed that there is no significant difference between the inhibitors used in Staphylokinase and Nattokinase production as p value was greater than 0.05 as shown in table 13.. Hence, Tukey method was not performed further due to insignificant difference.



(A) (B)

Fig 16: Result of Effect of Inhibitors on Invitro clot lysis of (A) Staphylokinase and (B) Nattokinase



(A) (B)

Fig 17: Result of Effect of Inhibitors on Anti-Thrombotic Activity of (A) Staphylokinase and (B) Nattokinase

8. Comparision between before and after optimization: fig 17 and fig 18

Table 13: ANOVA to Study the Effect of Inhibitors on Enzyme Production						
Staphylokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	78.46222	2	39.23111	2.434195	0.168251	5.143253
Within Groups	96.7	6	16.11667			
Total	175.1622	8				
Nattokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	100.7582	2	50.3791	3.030972	0.123084	5.143253
Within Groups	99.7286	6	16.62143			
Total	200.4868	8				

9. Comparision between Staphylokinase and Nattokinase Production Before and After Optimization: It was observed that the invitro clot lysis and anti-thrombotic activity increased after optimization of both the enzyme at optimum conditions provided. Staphylokinase production was more as compared to Nattokinase production. The statistical analysis revealed that for Staphylokinase there is significant difference in the conditions given before and after optimization as p value is less than 0.05 as shown in table 14 and table 15, while for Nattokinase it was found that there is no significant difference observed statistically.

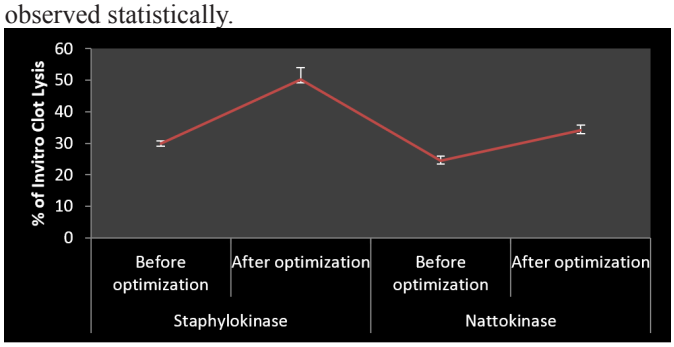


Fig 18: Comparison between Invitro clot Lysis of Staphylokinase and Nattokinase Before and After optimization

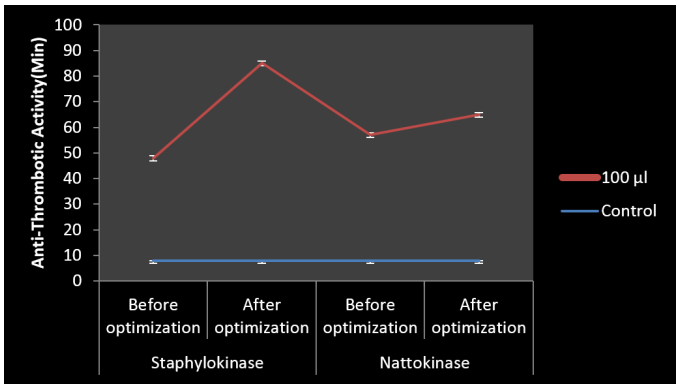


Fig 19: Comparision between Anti-Thrombotic Activity of Staphylokinase and Nattokinase Before and After optimization

Table 14: ANOVA for Comparision between EnzymeProduction Before and After Optimization						
Staphylokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	378.3025	1	378.3025	198.8449	0.004991	18.51282
Within Groups	3.805	2	1.9025			

Total	382.1075	3				
Nattokinase						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	88.36	1	88.36	3.583131	0.198889	18.51282
Within Groups	49.32	2	24.66			
Total	137.68	3				

Table 15: Tukey Method for Staphylokinase Production		
X1 -X2	20.3>1.9368	difference between 1 and 2 is sig at α=0.05

4. CONCLUSION

From the present study it can be concluded that the Staphylokinase and Nattokinase both poses strong fibrinolytic and anti-thrombotic activity. Staphylokinase is proved to be more potent and efficient as compared to Nattokinase. It is also seen that as the clot lysis property increase it also increases the clotting time. Hence can be proved as an efficient clot buster. These organisms can be further used for the production of thrombolytic agents which can act as a clot buster and the production cost can be decreased by using cheap substrate. The fibrinolytic enzymes obtained can be used as a food supplement and further studies can be done to assay its application as food additives and the advantage of it over the other commericially available thrombolytic agents.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

No conflict of Interest is declared here

7. REFERENCES

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